Reference: *Biol. Bull.*, 139: 222–228. (August, 1970)

NATURAL AND SYNTHETIC MATERIALS WITH INSECT HORMONE ACTIVITY. 5. SPECIFIC JUVENILE HORMONE EFFECTS IN ALIPHATIC SESQUITERPENES

K. SLÁMA, K. HEJNO, V. JAROLÍM, AND F. ŠORM

Institute of Entomology, and Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague

Insects belonging to different taxonomical groups often show great differences in their sensitivities to juvenile hormone analogues. For example, some compounds, such as the esters of monocyclic sesquiterpenes, have substantial activity on the hemipteran insects of the family Pyrrhocoridae, but little or no activity for most other insects including other families of the Hemiptera (Sláma, Suchý and Šorm, 1968). By contrast, there are many compounds, such as methylenedioxyphenylethers of geraniol (Bowers, 1969), which show great activity on pupae of Coleoptera but only slight activity on hemipterans.

In previous studies of juvenile hormone activity of farnesenic acid esters (Sláma, Romaňuk and Šorm, 1969) we have noticed that minor changes in chemical structure, such as the introduction of hydrogen chloride across the 6,7 and 10,11 double bonds, may lead to as much as 10,000-fold increase in activity for the bug *Pyrrhocoris apterus* with a simultaneous 100-fold loss of activity for the beetle *Tenebrio molitor*. Other studies on hemipteran insects (Suchý, Sláma and Šorm, 1968) have documented the considerable differences between the families or higher taxonomic groups in their response to individual juvenile hormone analogues.

In the present investigation we have explored the phenomenon in further detail. To this end we have determined the juvenile hormone activity of each of a series of fifteen synthetic esters of aliphatic sesquiterpenic acids when tested on eight genera of insects including one or more representatives of three families of Hemiptera and two families of Coleoptera. Special attention was centered on those features of molecular structure which enhance or detract from hormonal activity for individual families and genera of insects.

Materials and Methods

Juvenile hormone activity was assayed on the following Hemiptera: *Pyrrhocoris apterus* L. and *Dysdercus* (*Paradysdercus*) *cingulatus* (Fabr.) (family Pyrrhocoridae); *Lygaeus equestris* L. (family Lygaeidae); *Graphosoma italicum* Müll., *Aelia acuminata* L., and *Eurygaster integriceps* Put. (family Pentatomidae). Each analogue was also assayed on the following Coleoptera: *Tenebrio molitor* L. (family Tenebrionidae) and *Dermestes vulpinus* Fabr. (family Dermestidae).

For topical assays the compounds were applied in a standard 1 μl drop of acetone on uninjured cuticle of freshly molted last instar larvae (Hemiptera) or freshly molted pupae (0–20 hrs) (Coleoptera). For injection assays on *Tenebrio*

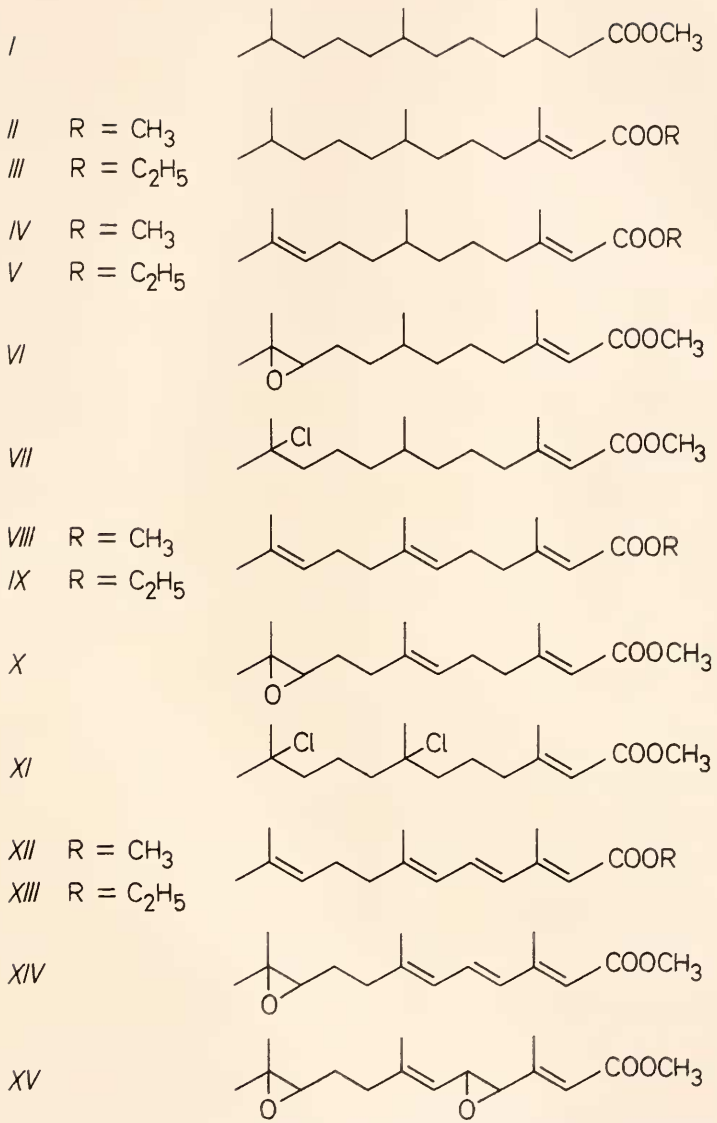

FIGURE 1. List of the compounds used for assays of juvenile hormone activity.

and *Dermestes* the compounds were injected in a 1 μl drop of olive oil into the body cavity of freshly molted pupae. The activity was determined according to the degree of retention of the larval (Hemiptera) or pupal (Coleoptera) characters after the next ecdysis.

The dose-response experiments performed on *Pyrrhocoris* and *Graphosoma* revealed that the whole range of activity from zero (formation of perfect normal adults) to maximum (formation of morphologically perfect supernumerary larval

instars) was realized with a 10-fold change in concentration. The reciprocal increase of larval epidermal patterns and decrease of adult patterns was linear when plotted against the logarithmic scale of concentrations. This allowed determination of a standard intermediate effect with minimum error. In the pupae of Coleoptera the range of activity from zero to maximum (extra-pupal instars) was realized with 100 to 1000-fold concentration change. The activity is given in "ID-50 Morph." units. This unit indicates the amount of substance in micrograms per specimen which produces under the above described conditions of application half-larval (Hemiptera) or half-pupal (Coleoptera) adultoids. The unit concentration occurs in the middle of the concentration range necessary for zero to maximum effect. The concentrations provoking the first signs of activity can easily be derived from the ID-50 values. For example, if the ID-50 for a hemipteran larva is 0.05, the first signs of activity occur at 0.01; so also, maximum activity would be attained by the application of approximately 0.1 μg. Each value in Table I represents a result of 4 to 5 tests at different concentrations, each concentration being assayed on 5 to 10 individuals.

The list of the compounds is presented in Figure 1. The compounds II to V, VIII, XII, and XIII were prepared by means of Wittig's reaction in which the aliphatic methyl ketones were treated with carbomethoxymethylene-triphenylphosphorane (for preparation of methyl esters) or carboethoxymethylene-triphenylphosphorane (for preparation of ethyl esters). This method yielded rather pure products.

The starting material for preparation of compounds II and III was hexahydropseudoionone, for IV and V citronellylacetone, for VIII geranylacetone, and for XII and XIII pseudoionone. Compound I was prepared by hydrogenation of VIII in presence of Pd/C catalyst. Compound VII was obtained from IV by addition of hydrogen chloride. The epoxides VI, X, and XIV were obtained from IV, VIII and XII after treatment with perphthalic acid. Similar technique was used to prepare the diepoxyderivative XV (two equivalents of perphthalic acid were used). Compounds IX and XI were kindly provided by Dr. M. Romaňuk.

All the synthesized compounds were purified by thin-layer or column chromatography on silica gel and their purity was checked by gas-liquid chromatography (with the exception of halogen-containing compounds). In some cases infra-red and mass spectrometry was used for further characterization.

Results

All the compounds studied were methyl or ethyl esters of aliphatic C_{15} terpenoid acids which differed in the amounts and positions of the double bonds. For easier orientation we have divided the compounds into the following groups:

Group **A**—Methyl ester of 3,7,11-trimethyl-dodecanoic acid (I) with fully saturated molecule.

B—Esters of 3,7,11-trimethyl-2-dodecenic acid (II, III) with one double bond.

C—Esters of 3,7,11-trimethyl-2,10-dodecadienoic acid (IV, V) with 10,11-epoxy (VI) and 11-chloro (VII) derivatives.

D—Esters of 3,7,11-trimethyl-2,6,10-dodecatrienoic acid (VIII, IX) with three double bonds including also 10,11-epoxy (X) and 7,11-dichloro (XI) derivatives.

E—Esters of 3,7,11-trimethyl-2,4,6,10-dodecatetraenoic acid (XII, XIII) with four double bonds including 10,11-epoxy (XIV) and 4,5,10,11-diepoxy derivatives (XV).

The juvenile hormone activity of simple esters

As summarized in Table I, the saturated ester (I) showed no detectable activity except when high concentrations were administered to pyrrhocorid bugs. By contrast, substantial activity was recorded for the esters (II, III) of group B

TABLE I

Juvenile hormone activity of the compounds listed in Figure 1. The values indicate ID-50 (Morph) units in μg per specimen

Family	Pyrrhocoridae		Lygaeidae	Pentatomidae			Tenebrionidae		Dermestidae	
Species	*Pyrrhocoris apterus*	*Dysdercus cingulatus*	*Lygaeus equestris*	*Graphosoma italicum*	*Aelia acuminata*	*Eurygaster integriceps*	*Tenebrio molitor*		*Dermestes vulpinus*	
Application	topical	topical	topical	topical	topical	topical	injection	topical	injection	topical
Compound No.										
I	100	100	>500	>1000	>1000	>1000	>1000	>1000	>1000	>1000
II	5	10	7	100	70	>100	1	10	1	10
III	5	10	5	50	50	—	1	7	0.5	5
IV	5	5	3	50	50	100	5	20	5	—
V	5	5	3	8	—	—	5	10	5	10
VI	0.5	0.07	0.5	1	4	—	3	10	3	9
VII	0.08	0.05	0.9	5	5	—	1	1	5	5
VIII	50	50	50	50	50	100	10	100	8	—
IX	50	30	40	50	30	100	5	100	5	—
X	3	1	—	10	5	20	10	—	—	—
XI	0.0008	0.005	0.009	30	1	30	1000	>1000	25	50
XII	3	1	5	3	5	20	500	>1000	100	—
XIII	3	1	—	1	5	20	500	>1000	100	—
XIV	0.8	0.7	1	1	7	5	100	>1000	—	—
XV	0.8	0.7	1	5	7	8	>1000	>1000	>500	—

The tested compounds contained approximately $\frac{2}{3}$ *trans* methyl-3 isomers.

which contain the conjugated double bond at C_2. These compounds showed a 10- to 20-fold increase in activity for the pyrrhocorids, and the appearance of low but definite activity for the pentatomids. In the case of the two species of Coleoptera, the increase in activity was at least a thousandfold.

With further increase in the number of double bonds the activity undergoes definite changes distinctive of each taxonomic group. The results may be summarized as follows: (i) In the Pyrrhocoridae and Lygaeidae the activity does not show any considerable variations with increasing degree of unsaturation, *i.e.*, critical doses are between 1 and 10 μg with the exception of farnesenic acid esters (VIII, IX) which are less active. (ii) The Pentatomidae show a continuous increase of juvenile activity with increasing degree of unsaturation; the difference is approximately 10 to 100-fold. (iii) Just the reverse is true for the coleopterans, *Tenebrio* and *Dermestes*, where the esters of group B (II, III) are the most active

and the highly unsaturated compounds of group E (XII, XIII) are 100 to 1000 times less active or completely inactive.

The effect of epoxidation

When the 10,11 double bond of the compounds IV and V is saturated by an oxirane ring (VI), hormonal activity is substantially increased in hemipteran insects and slightly increased also in the beetles. The increase is smaller in XIV where the oxirane ring is placed across the 10,11 double bond of the highly unsaturated compound XII. The diepoxy-ester (XV) with both 10,11 and 4,5 double bonds epoxidated has approximately the same activity as the monoepoxy-ester (XIV), suggesting that the oxirane ring in the position 4,5 does not increase the activity as that in the position 10,11.

The effect of hydrochlorination

Substitution of the 10,11 double bond in compounds IV and V by hydrogen chloride has similar effects on hormonal activity as described above for the corresponding epoxy-derivative. When both the 10,11 and 6,7 double bonds of compounds VIII and IX are saturated by hydrogen chloride (XI) there occurs an

TABLE II

Summary of the relationships between the double bonds, their substitutions by hydrogen chloride and epoxide, and juvenile hormone activity

	Pyrrhocoridae Lygaeidae	Pentatomidae	Tenebrionidae Dermestidae
Increasing degree of unsaturation	little or no change	increase	decrease
10, 11 substitution	large increase	increase	slight increase
6,7 substitution	large increase	slight decrease	large decrease
6,7 and 10,11 substitution	enormous increase	slight increase	large decrease

enormous increase in activity for pyrrhocorid and lygaeid bugs, whereas little change in activity is observed for pentatomids. Equally impressive is the great decline in activity for the beetles. Since the monohydrochlorinated compound VII is highly active on beetles, the 6,7 double bond and especially the status of C_7 seem crucial. Thus, when the C_7 is attached to a halogen atom or is bound to an oxygen atom of an epoxide, the compounds are very active on pyrrhocorids and very inactive on the beetles. By contrast, in the pentatomid bugs the activity is little influenced by changes at C_7.

The effect of stereochemical isomers

The *cis* and *trans* isomers of compounds II and VII were isolated and tested on *Pyrrhocoris, Dysdercus,* and *Graphosoma* by topical assays and on *Tenebrio* by both topical and inject assays. The *trans* isomers were found approximately 10 times more active than the *cis* isomers in all the hemipterans. In *Tenebrio* there were large variations in activity but, in general, the *trans* isomers appeared to be 5 to 100 times more active than the *cis* isomers.

The effect of methyl and ethyl ester radicals

In the present study we found rather small difference in the activity of methyl and ethyl esters on pyrrhocorids, and relatively small activity increase of ethyl esters over the corresponding methyl esters in other insects studied. Present indications are that the ethyl esters cause a greater increase in activity in compounds where the 10,11 double bond is saturated by hydrogen chloride or epoxide.

Discussion

Jarolím, Hejno, Selnal and Šorm (1969) have described the juvenile hormone activity of the compounds listed in Figure 1 on larvae and pupae of *Galleria mellonella* (Lepidoptera, family Pyralidae). It appears that the action of these compounds on *Galleria* is in many respects similar to that which we have found on pupae of the beetles, *Tenebrio* and *Dermestes*.

It is known from the literature that there are certain structural features of juvenile hormone analogues which produce a general increase or decrease of activity in all species. Our results have confirmed these generalizations which can be summarized as follows: (i) higher activity of stereochemical *trans* isomers (Yamamoto and Jacobson, 1962; Röller and Dahm, 1969; Wigglesworth, 1969a; Sláma *et al.*, 1969; (ii) necessity of the 2,3 double bond in aliphatic terpenes; (iii) loss of activity after introduction of very polar groups such as hydroxyls (Suchý *et al.*, 1968); (iv) increased activity after addition of 10,11-epoxy group (Bowers, Thompson and Uebel, 1965; Röller and Dahm, 1969; Ratuský, Sláma and Šorm, 1969; Wigglesworth, 1969a, 1969b; Jarolím *et al.*, 1969); (v) increased activity after additions of hydrogen chloride at the 10,11 double bond (Law, Yuan and Williams, 1966; Romaňuk, Sláma and Šorm, 1967; Sláma *et al.*, 1969; Wigglesworth, 1969a); (vi) higher activity of ethyl esters (Law *et al.*, 1966; Röller and Dahm, 1969; Sláma *et al.*, 1969; Wigglesworth, 1969a).

As indicated in the present study, there are certain chemical changes which lead to predictable increase or decrease of the juvenile activity for individual families and orders of insects. These have been summarized in Table II. The structure-activity relationships of this type are not common in the literature for they require large biological screenings on representatives of several insect groups. We have already mentioned that the degree of unsaturation and the chemical configuration at C_7 are among the factors determining selective action in esters of aliphatic sesquiterpenes. Both these factors seem to be associated with one and the same biological mechanism since each group of insects which shows specific responses to the degree of unsaturation is also sensitive to substitutions at C_7.

We suspect that this type of information will be helpful in the preparation of new synthetic juvenile hormone analogues with more or less selective pesticide effects.

Acknowledgment is gratefully given to Professor C. M. Williams who provided helpful comments on the typescript and to Mrs. Pichlová, Mrs. Piščáková and Mrs. Zdeňková for their technical assistance.

Summary

The juvenile hormone activity of ethyl or methyl esters of aliphatic sesquiterpenic acids with 0 to 4 double bonds was tested on 8 species of insects belonging to 5 families of Hemiptera and Coleoptera. Special attention was paid to the addition of hydrogen chloride or epoxide groups on or across the double bonds.

Certain chemical changes in the molecule appear to cause a general increase of the activity in all species studied. These are: the presence of 2,3 unsaturation conjugated with the carboxyl group; the *trans* stereochemical position of the C-3 methyl; an introduction of 10,11 epoxide or hydrochloride; and esterification with ethyl rather than with methyl.

There are also chemical changes which lead to genus- or family-specific variations in juvenile hormone activity. With increasing amount of unsaturation the activity either remains almost unaffected (pyrrhocorid bugs) or increases (pentatomid bugs) or decreases considerably (tenebrionid and dermestid beetles). The addition of hydrogen chloride or epoxide to the 6,7 double bond causes enormous increase in the activity in the Pyrrhocoridae and Lygaeidae, no considerable change in the Pentatomidae, and great decreases in the beetles and Lepidoptera.

LITERATURE CITED

Bowers, W. S., 1969. Juvenile hormone: activity of aromatic terpenoid ethers. *Science,* **154**: 323–325.

Bowers, W. S., M. J. Thompson and E. C. Uebel, 1965. Juvenile and gonadotropic hormone activity of 10,11 epoxyfarnesenic acid methylester. *Life Sciences,* **4**: 2323–2331.

Jarolím, V., K. Hejno, F. Sehnal and F. Šorm, 1969. Natural and synthetic materials with insect hormone activity. 8. Juvenile activity of the farnesane-type compounds on *Galleria mellonella*. *Life Sciences,* **8**: 831–841.

Law, J. H., C. Yuan and C. M. Williams, 1966. Synthesis of a material with high juvenile hormone activity. *Proc. Nat. Acad. Sci.,* **55**: 576–578.

Ratuský, J., K. Sláma and F. Šorm, 1969. Natural and synthetic materials with insect hormore activity. 6. Juvenile hormone effects of some alkyl-ethers derived from aliphatic β-hydroxy acids. *J. Stored Prod. Res.,* **5**: 111–117.

Röller, H., and K. H. Dahm, 1968. The chemistry and biology of juvenile hormone. *Recent Prog. Hormone Res.,* **24**: 651–680.

Romaňuk, M., K. Sláma and F. Šorm, 1967. Constitution of a compound with a pronounced juvenile hormone activity. *Proc. Nat. Acad. Sci.,* **57**: 349–352.

Sláma, K., M. Suchý and F. Šorm, 1968. Natural and synthetic materials with insect hormone activity. 3. Juvenile hormone activity of derivatives of p-(1,5-dimethylhexyl) benzoic acid. *Biol. Bull.,* **134**: 154–159.

Sláma, K., M. Romaňuk and F. Šorm, 1969. Natural and synthetic materials with insect hormone activity. 2. Juvenile hormone activity of some derivatives of farnesenic acid. *Biol. Bull.,* **136**: 91–95.

Suchý, K., K. Sláma and F. Šorm, 1968. Insect hormone activity of p-(1,5-dimethylhexyl) benzoic acid derivatives in *Dysdercus* species. *Science,* **162**: 582–583.

Wigglesworth, V. B., 1969a. Chemical structure and juvenile hormone activity: comparative tests on *Rhodnius prolixus*. *J. Insect Physiol.,* **15**: 73–94.

Wigglesworth, V. B., 1969b. Chemical structure and juvenile hormone activity. *Nature,* **221**: 190–191.

Yamamoto, R. T., and M. Jacobson, 1962. Juvenile hormone activity of isomers of farnesol. *Nature,* **196**: 908–909.